# METHOD FOR PREPARING NUCLEATED BLOOD IN BULK FOR CLASS DEMONSTRATION.

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No book on microscopical technique which I have been able to consult gives a method for preparing blood in bulk.

For class demonstration it is obvious that by having on hand ready prepared material the work will be greatly facilitated and a uniformity of result assured which could not be expected from the faulty manipulations of untrained students to whom blood is usually given for study early in their histological course.

It is much more convenient for the teacher to dispense his preparation from a small vial than to be compelled to make "smears" for a large class. "Smears" are also often unsatisfactory by reason of agglutination or crenation of the corpuscles, excess of serum and the formation of fibrin and much care is required in their proper fixation, by the usual method of heat, in order that the result be not disastrous.

These considerations led me to try and work out a method which would allow of the staining and keeping of nucleated blood in bulk ready for distribution to the class and so fixed that there should be but little distortion of the corpuscles.

The red blood cell is a delicate structure and some care in its manipulation is required.

If the steps of the method are strictly followed one may be confident of a successful issue.

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Chloroform the animal selected; a large frog is probably the most convenient; open the thorax, puncture the aorta and allow the blood to flow directly into a small glass jar, with ground glass stopper, containing a one per cent (1%) aqueous solution of osmic acid. The solution should be largely in excess of the

amount of blood, at least fifty times as great. The vessel is now closed and set aside for several hours in which time the blood cells will have become thoroughly fixed and hardened and have settled in a thin layer at the bottom.

Decant the supernatant fluid and add distilled water, gently agitating the vessel until the blood is thoroughly mixed with the water. Again decant after sedimentation has taken place or filter rapidly through very thin filter paper and wash off the filtrate in a small quantity of distilled water.

Next add Böhmer's haematoxylin diluted one-half with distilled water. Use no more of this mixture than enough to promote quick and thorough admixture with the water containing the blood. After a few moments staining filter as before, wash the filtrate from the paper by agitating in a large dish of distilled water and set the vessel aside for an hour or more in order that the nuclei of the cells may be well differentiated.

Dehydration is now accomplished by running the blood through various strengths of alcohol beginning with seventy per cent (70%) and ending with absolute, filtration or decantation being practiced with each step. Care must be taken not to use too small a quantity of alcohol or the cells will not be well dehydrated.

Clear in carbol-xylol (carbolic acid one part, xylol three parts), allow the blood to settle in a large test tube or conical glass, draw off as much of the fluid as possible with a bulb pippete and add thin xylol balsam.

Keep in a well stoppered bottle and when wanted for use shake until the blood is thoroughly mixed with the balsam, with a small glass rod transfer a drop to a clean slide and superimpose a cover glass. A neat and permanent preparation is the result.